

inducing direct somatic embryogenesis and organogenesis in monocotyledonous plant cells and rapidly regenerating fertile monocotyledonous plants; and for inducing somatic embryogenesis in monocotyledonous callus cells, suspension cells, or microspore-derived embryos, and rapidly regenerating fertile monocotyledonous plants. Fertile monocotyledonous plants thereby produced are also provided. --

In the claims:

Please cancel claims 1 - 106 of the application currently on file and replace with new claims 107 - 207 as follows:

- 107. A process for inducing direct somatic embryogenesis in monocotyledonous plant cells and rapidly regenerating fertile monocotyledonous plants, comprising the steps of:
- (a) culturing embryogenic monocotyledonous plant cells under conditions conducive to direct formation of primary embryos without an intervening callus stage, at least until at least one primary embryo reaches the globular developmental stage; and one of the following steps selected from:
    - (b) culturing one or more of the primary embryos from step (a) under conditions conducive to regeneration of plantlets from the primary embryos, and culturing the primary embryo in or on a regeneration medium;
    - (c) culturing one or more of the primary embryos at the globular developmental stage and no longer than the coleoptilar stage from step (a) under conditions conducive to induction of secondary embryo formation, at least until secondary embryogenesis is detected, and culturing one or more of the secondary embryos under conditions conducive to regeneration of plantlets from the secondary embryos; or
    - (d) culturing one or more of the primary embryos at the globular developmental stage and no longer than the coleoptilar stage from step (a) under conditions conducive to induction of organogenesis, at least until adventitious shoots are detected; and culturing the adventitious shoots under conditions conducive to regeneration of plantlets.

108. The process of claim 107, wherein step (a) further comprises culturing the embryogenic cells in or on a culture medium comprising auxin, cytokinin and polyamine in amounts effective to cause direct formation of primary embryos without an intervening callus stage, the auxin being present in greater proportion than the cytokinin.

109. The process of claim 108, wherein, in step (a), the ratio of auxin to cytokinin in the culture medium is from about 5  $\mu\text{M}$  auxin per 1  $\mu\text{M}$  cytokinin to about 20  $\mu\text{M}$  auxin per 1  $\mu\text{M}$  cytokinin.

110. The process of claim 109, wherein, in step (a) the culture medium includes the plant growth regulators:

- i) from about 15  $\mu\text{M}$  auxin to about 45  $\mu\text{M}$  auxin;
- ii) from about 15  $\mu\text{M}$  polyamine to about 45  $\mu\text{M}$  polyamine; and
- iii) from about 1  $\mu\text{M}$  cytokinin to about 5  $\mu\text{M}$  cytokinin.

111. The process of claim 108, wherein, in step (a), the ratio of auxin to cytokinin in the culture medium is about 14  $\mu\text{M}$  auxin per 1  $\mu\text{M}$  cytokinin.

112. The process of claim 111, wherein, in step (a), the culture medium includes the plant growth regulators of:

- i) about 30  $\mu\text{M}$  auxin;
- ii) about 30  $\mu\text{M}$  polyamine; and
- iii) about 2  $\mu\text{M}$  cytokinin.

113. The process of claim 108, wherein, in step (a), the culture medium is DSEM medium.

114. The process of claim 110, wherein steps (a) and (b) are conducted, and the regeneration medium is MS medium.

115. The process of claim 110, wherein steps (a) and (c) are conducted, and further comprise culturing the primary embryo in or on a culture medium comprising auxin, cytokinin, and polyamine in amounts effective to cause induction of secondary embryo formation, the cytokinin being present in greater proportion than the auxin.

116. The process of claim 110, wherein steps (a) and (d) are conducted, and further comprise culturing the primary embryo in or on a culture medium comprising auxin, cytokinin, and polyamine in amounts effective to cause induction of organogenesis, the cytokinin being present

in greater proportion than the auxin.

117. The process of claim 114, further comprising, before step (b), the step of culturing the primary embryo under conditions conducive to germination of the primary embryos until germination of at least one of the primary embryos commences.

118. The process of claim 117, wherein the germination step comprises culturing the primary embryo in or on a culture medium which comprises polyamine in an amount effective to cause germination of the primary embryos, and which is essentially free of either auxin or cytokinin.

119. The process of claim 118, wherein the culture medium comprises from about 15  $\mu\text{M}$  polyamine to about 45  $\mu\text{M}$  polyamine.

120. The process of claim 118, wherein the culture medium comprises about 30  $\mu\text{M}$  polyamine.

121. The process of claim 118, wherein the germination step comprises culturing the primary embryo in or on GEM medium.

122. The process of claim 114, further comprising the step of culturing the plantlets under conditions conducive to induction of root formation until the plantlets form roots.

123. The process of claim 122, further comprising the step of transplanting the plantlets to soil and growing them to maturity.

124. The process of claim 123, wherein the embryogenic cells are Poaceae embryogenic cells, wherein the cells are selected from the genera consisting of *Triticum*, *Hordeum*, *Secale*, *Avena*, *Zea*, *Oryza*, *Sorghum*, *Pennisetum*, *Saccharum*, *Dactylis*, *Bromus*, and *Lolium*; or Liliaceae embryogenic cells, wherein the cells are selected from the genus *Allium*.

125. The process of claim 124, wherein the embryogenic cells are selected from the group consisting of *Hordeum vulgare*, *Triticum aestivum*, *Triticum durum*, *Triticum monococcum*, *Triticum urartu*, *Secale cereale*, *Avena sativa*, *Zea mays*, *Sorghum bicolor* and *Triticum durum amphiploids* embryogenic cells.

126. The process of claim 124, wherein the embryogenic cells of step (a) are scutella cells.

127. The process of claim 124, wherein the embryogenic cells of step (a) are scutella cells free of a germ.

128. The process of claim 127, which further includes, after step (a), cutting the scutellum

carrying the primary embryo into a plurality of pieces prior to culturing in step (b).

129. The process of claim 128, wherein the scutellum carrying the primary embryo is cut into two to four pieces.

130. The process of claim 124, wherein step (a) further comprises the step of introducing foreign DNA into the embryogenic cells or primary embryo so that the foreign DNA becomes stably integrated into the genome of the cells.

131. The process of claim 130, wherein the foreign DNA is introduced into the embryogenic cells or primary embryo by particle bombardment or by *Agrobacterium*-mediated transformation.

132. The process of claim 131, wherein the foreign DNA is introduced into the embryogenic cells or primary embryo in step (a) during the development of the primary embryo.

133. The process of claim 132, wherein the foreign DNA is introduced into the embryogenic cells between zero to five days after commencement of tissue culture.

134. The process of claim 132, wherein the foreign DNA is introduced into the embryogenic cells or the primary embryo after two days following commencement of tissue culture.

135. The process of claim 132, wherein after the foreign DNA has been introduced, the embryogenic cells or primary embryo are transferred to a media for steps (a) and (b) which includes a selective agent to identify a transformed plant cell that has incorporated the foreign DNA.

136. The process of claim 135, wherein transformed plant cells are cultured in media to support regeneration of transformants.

137. The process of claim 136, which further comprises confirming expression of the foreign DNA in the transformed plants by one or both of polymerase chain reaction and Southern blot analyses.

138. The process of claim 115, wherein, in step (c), the ratio of auxin to cytokinin in the culture medium is from about 0.05  $\mu\text{M}$  auxin per 1  $\mu\text{M}$  cytokinin to about 0.2  $\mu\text{M}$  auxin per 1  $\mu\text{M}$  cytokinin.

139. The process of claim 138, wherein, in step (c), the culture medium includes the plant growth regulators:

- i) from about 5  $\mu\text{M}$  auxin to about 15  $\mu\text{M}$  auxin;

- ii) from about 15  $\mu$ M polyamine to about 45  $\mu$ M polyamine; and
  - iii) from about 50  $\mu$ M cytokinin to about 200  $\mu$ M cytokinin.
140. The process of claim 115, wherein, in step (c) the ratio of auxin to cytokinin is about 0.1  $\mu$ M auxin per 1.0  $\mu$ M cytokinin.
141. The process of claim 140, wherein, in step (c), the culture medium includes the plant growth regulators of:
- i) about 11  $\mu$ M auxin;
  - ii) about 30  $\mu$ M polyamine; and
  - iii) about 110  $\mu$ M cytokinin.
142. The process of claim 115, wherein, in step (c), the culture medium is SEM medium.
143. The process of claim 142, wherein step (c) comprises culturing the secondary embryo in or on a regeneration medium.
144. The process of claim 143, wherein the regeneration medium is MS medium.
145. The process of claim 143, further comprising, before step (c), the step of culturing the secondary embryo under conditions conducive to germination of the secondary embryos until germination of at least one of the secondary embryos commences.
146. The process of claim 145, wherein the germination step comprises culturing the secondary embryo in or on a culture medium which comprises polyamine in an amount effective to cause germination of the secondary embryos, and which is essentially free of either auxin or cytokinin.
147. The process of claim 146, wherein the culture medium comprises from about 15  $\mu$ M polyamine to about 45  $\mu$ M polyamine.
148. The process of claim 146, wherein the culture medium comprises about 30  $\mu$ M polyamine.
149. The process of claim 146, wherein the germination step comprises culturing the secondary embryo in or on GEM medium.
150. The process of claim 143, further comprising the step of culturing the plantlets under conditions conducive to induction of root formation until the plantlets form roots.
151. The process of claim 150, further comprising the step of transplanting the plantlets to soil

and growing them to maturity.

152. The process of claim 151, wherein the embryogenic cells are Poaceae embryogenic cells, wherein the cells are selected from the genera consisting of *Triticum*, *Hordeum*, *Secale*, *Avena*, *Zea*, *Oryza*, *Sorghum*, *Pennisetum*, *Saccharum*, *Dactylis*, *Bromus*, and *Lolium*; or Liliaceae embryogenic cells, wherein the cells are selected from the genus *Allium*.

153. The process of claim 152, wherein the embryogenic cells are selected from the group consisting of *Hordeum vulgare*, *Triticum aestivum*, *Triticum durum*, *Triticum monococcum*, *Triticum urartu*, *Secale cereale*, *Avena sativa* and *Triticum durum amphiploids* embryogenic cells.

154. The process of claim 152, wherein the embryogenic cells of step (a) are scutella cells.

155. The process of claim 152, wherein the embryogenic cells of step (a) are scutella cells free of a germ.

156. The process of claim 155, which further includes, after step (a), cutting the scutellum carrying the primary embryo into a plurality of pieces before culturing in step (c).

157. The process of claim 156, wherein the scutellum carrying the primary embryo is cut into two to four pieces.

158. The process of claim 156, which further comprises, before step (c), the step of cutting the primary embryo carrying the secondary embryo into a plurality of pieces, or cutting a germinating leaf if developed, to obtain a high frequency of germination of secondary embryo.

159. The process of claim 158, wherein the primary embryos carrying the secondary embryo is cut into two pieces.

160. The process of claim 152, wherein step (a) further comprises the step of introducing foreign DNA into the embryogenic cells or the primary embryo so that the foreign DNA becomes stably integrated into the genome of the cells.

161. The process of claim 160, wherein the foreign DNA is introduced into the embryogenic cells or primary embryo by particle bombardment or by *Agrobacterium*-mediated transformation.

162. The process of claim 161, wherein the foreign DNA is introduced into the embryogenic cells or the primary embryo in step (a) during the development of the primary embryo.

163. The process of claim 162, wherein the foreign DNA is introduced into the embryogenic

cells or the primary embryo between zero to five days after commencement of tissue culture.

164. The process of claim 162, wherein the foreign DNA is introduced into the embryogenic cells or the primary embryo after two days following commencement of tissue culture.

165. The process of claim 162, wherein after the foreign DNA has been introduced, the embryogenic cells or primary embryo are transferred to a media for step (c), and optionally for step (a), which includes a selective agent to identify a transformed plant cell that has incorporated the foreign DNA.

166. The process of claim 165, wherein transformed plant cells are cultured in media to support regeneration of transformants.

167. The process of claim 166, which further comprises confirming expression of the foreign DNA in the transformed plants by one or both of polymerase chain reaction and Southern blot analyses.

168. The process of claim 116, wherein, in step (d), the ratio of auxin to cytokinin in the culture medium is from about 0.05  $\mu\text{M}$  auxin per 1  $\mu\text{M}$  cytokinin to about 0.2  $\mu\text{M}$  auxin per 1  $\mu\text{M}$  cytokinin.

169. The process of claim 168, wherein, in step (d), the culture medium includes the plant growth regulators:

- i) from about 5  $\mu\text{M}$  auxin to about 15  $\mu\text{M}$  auxin;
- ii) from about 15  $\mu\text{M}$  polyamine to about 45  $\mu\text{M}$  polyamine; and
- iii) from about 50  $\mu\text{M}$  cytokinin to about 200  $\mu\text{M}$  cytokinin.

170. The process of claim 116, wherein, in step (d) the ratio of auxin to cytokinin is about 0.1  $\mu\text{M}$  auxin per 1.0  $\mu\text{M}$  cytokinin.

171. The process of claim 170, wherein, in step (d), the culture medium includes the plant growth regulators of:

- i) about 11  $\mu\text{M}$  auxin;
- ii) about 30  $\mu\text{M}$  polyamine; and
- iii) about 110  $\mu\text{M}$  cytokinin.

172. The process of claim 116, wherein, in step (d), the culture medium is SEM medium.

173. The process of claim 169, wherein step (d) comprises culturing the new shoots in or on a

regeneration medium.

174. The process of claim 173, wherein the regeneration medium is MS medium.

175. The process of claim 173, further comprising the step of culturing the plantlets and shoots under conditions conducive to induction of root formation until the plantlets form roots.

176. The process of claim 175, comprising the further step of transplanting the plantlets to soil and growing them to maturity.

177. The process of claim 176, wherein the embryogenic cells are Poaceae embryogenic cells, wherein the cells are selected from the genera consisting of *Triticum*, *Hordeum*, *Secale*, *Avena*, *Zea*, *Oryza*, *Sorghum*, *Pennisetum*, *Saccharum*, *Dactylis*, *Bromus*, and *Lolium*; or Liliaceae embryogenic cells, wherein the cells are selected from the genus *Allium*.

178. The process of claim 177, wherein the embryogenic cells are selected from the group consisting of *Zea mays* and *Sorghum bicolor*.

179. The process of claim 177, wherein the embryogenic cells of step (a) are scutella cells.

180. The process of claim 177, wherein the embryogenic cells of step (a) are scutella cells free of a germ.

181. The process of claim 180, which further includes, after step (a), cutting the scutellum carrying the primary embryo into a plurality of pieces before culturing in step (d).

182. The process of claim 181, wherein the scutellum carrying the primary embryo is cut into two to four pieces.

183. The process of claim 177, wherein step (a) further comprises introducing foreign DNA into the embryogenic cells or the primary embryo so that the foreign DNA becomes stably integrated into the genome of the cells.

184. The process of claim 183, wherein the foreign DNA is introduced into the embryogenic cells or primary embryo by particle bombardment or by *Agrobacterium*-mediated transformation.

185. The process of claim 184, wherein the foreign DNA is introduced into the embryogenic cells or primary embryo in step (a) during the development of the primary embryo.

186. The process of claim 185, wherein the foreign DNA is introduced into the embryogenic cells between zero to five days after commencement of tissue culture.

187. The process of claim 185, wherein the foreign DNA is introduced into the embryogenic



cells or the primary embryo after two days following commencement of tissue culture.

188. The process of claim 185, wherein after the foreign DNA has been introduced, the embryogenic cells or primary embryo are transferred to a media for steps (a) and (d) which includes a selective agent to identify a transformed plant cell that has incorporated the foreign DNA.

189. The process of claim 188, wherein transformed plant cells are cultured in media to support regeneration of transformants.

190. The process of claim 189, which further comprises confirming expression of the foreign DNA in the transformed plants by one or both of polymerase chain reaction and Southern blot analyses.

191. A process for inducing somatic embryogenesis in monocotyledonous callus cells, suspension cells, or microspore-derived embryos, and rapidly regenerating fertile monocotyledonous plants, comprising the steps of:

(a) culturing embryogenic monocotyledonous callus cells, suspension cells or microspore-derived embryos in or on a culture medium comprising auxin, cytokinin, and polyamine in amounts effective to cause induction of embryo formation, the cytokinin being present in greater proportion than the auxin, at least until at least one embryo reaches the globular developmental stage; and

(b) cultivating the one or more globular-stage embryos from step (a) under conditions conducive to regeneration of plantlets.

192. The process of claim 191, wherein, in step (a), the ratio of auxin to cytokinin in the culture medium is from about 0.05  $\mu\text{M}$  auxin per 1  $\mu\text{M}$  cytokinin to about 0.2  $\mu\text{M}$  auxin per 1  $\mu\text{M}$  cytokinin.

193. The process of claim 192, wherein, in step (a), the culture medium includes the plant growth regulators:

- i) from about 5  $\mu\text{M}$  auxin to about 15  $\mu\text{M}$  auxin;
- ii) from about 15  $\mu\text{M}$  polyamine to about 45  $\mu\text{M}$  polyamine; and
- iii) from about 50  $\mu\text{M}$  cytokinin to about 200  $\mu\text{M}$  cytokinin.

194. The process of claim 191, wherein, in step (a) the ratio of auxin to cytokinin is about 0.1

$\mu\text{M}$  auxin per 1.0  $\mu\text{M}$  cytokinin.

195. The process of claim 194, wherein, in step (a), the culture medium includes the plant growth regulators:

- i) about 11  $\mu\text{M}$  auxin;
- ii) about 30  $\mu\text{M}$  polyamine; and
- iii) about 110  $\mu\text{M}$  cytokinin.

196. The process of claim 191, wherein, in step (a), the culture medium is SEM medium.

197. The process of claim 193, wherein step (b) comprises culturing the embryo in or on a regeneration medium.

198. The process of claim 197, wherein the regeneration medium is MS medium.

199. The process of claim 197, further comprising the step of (c) culturing the plantlets under conditions conducive to induction of root formation until the plantlets form roots.

200. The process of claim 199, further comprising the step of (d) transplanting the plantlets to soil and growing them to maturity.

201. The process of claim 200, wherein the embryogenic monocotyledonous callus cells, suspension cells or microspore-derived embryos are of Poaceae and are selected from the genera consisting of *Triticum*, *Hordeum*, *Secale*, *Avena*, *Zea*, *Oryza*, *Sorghum*, *Pennisetum*, *Saccharum*, *Dactylis*, *Bromus*, and *Lolium*; or of Liliaceae and from the genus *Allium*.

202. The process of claim 201, wherein the embryogenic monocotyledonous callus cells, suspension cells or microspore-derived embryos are selected from the group consisting of *Hordeum vulgare*, *Triticum aestivum*, *Triticum durum*, *Triticum monococcum*, *Triticum urartu*, *Secale cereale*, *Avena sativa*, *Zea mays*, *Sorghum bicolor* and *Triticum durum amphiploids*.

203. The process of claim 201, which further comprises, before step (a), introducing foreign DNA into the embryogenic monocotyledonous callus cells, suspension cells or microspore-derived embryos so that the foreign DNA becomes stably integrated into the genome of the cells or embryos.

204. The process of claim 203, wherein the foreign DNA is introduced into the embryogenic monocotyledonous callus cells, suspension cells or microspore-derived embryos by particle bombardment or by *Agrobacterium*-mediated transformation.